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Norovirus Transmission between Hands, Gloves, Utensils, and Fresh Produce during Simulated Food Handling

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Human noroviruses (HuNoVs), a leading cause of food-borne gastroenteritis worldwide, are easily transferred via ready-to-eat (RTE) foods, often prepared by infected food handlers. In this study, the transmission of HuNoV and murine norovirus (MuNoV) from virus-contaminated hands to latex gloves during gloving, as well as from virus-contaminated donor surfaces to recipient surfaces after simulated preparation of cucumber sandwiches, was inspected. Virus transfer was investigated by swabbing with polyester swabs, followed by nucleic acid extraction from the swabs with a commercial kit and quantitative reverse transcription-PCR. During gloving, transfer of MuNoV dried on the hand was observed 10/12 times. HuNoV, dried on latex gloves, was disseminated to clean pairs of gloves 10/12 times, whereas HuNoV without drying was disseminated 11/12 times. In the sandwich-preparing simulation, both viruses were transferred repeatedly to the first recipient surface (left hand, cucumber, and knife) during the preparation. Both MuNoV and HuNoV were transferred more efficiently from latex gloves to cucumbers (1.2% ± 0.6% and 1.5% ± 1.9%) than vice versa (0.7% ± 0.5% and 0.5% ± 0.4%). We estimated that transfer of at least one infective HuNoV from contaminated hands to the sandwich prepared was likely to occur if the hands of the food handler contained 3 $\log_{10}$ or more HuNoVs before gloving. Virus-contaminated gloves were estimated to transfer HuNoV to the food servings more efficiently than a single contaminated cucumber during handling. Our results indicate that virus-free food ingredients and good hand hygiene are needed to prevent HuNoV contamination of RTE foods.

The effective transmission routes of human noroviruses (HuNoVs) are one of the major reasons why these viruses are recognized as the most common nonbacterial cause of gastroenteritis worldwide (1). HuNoVs spread via the fecal-oral route among humans but can also easily be transmitted to food via inanimate and animate surfaces, such as food preparation equipment and human hands (2, 3). In addition, food such as vegetables and soft fruit can be contaminated earlier in the food chain, e.g., via virus-contaminated irrigation water (2, 4). Once in food ingredients, HuNoV can probably persist on food for extended periods under frozen and cooled conditions, as well as at room temperature, as was shown in HuNoV surrogate studies (5, 6). Several attributes of HuNoV, such as a high virus load in the vomit and feces of infected individuals, a prolonged virus-shedding time, a small infective dose of the virus, and high environmental stability, all facilitate virus transmission from the environment and foods to humans (7).

Virus contamination during preparation of ready-to-eat (RTE) foods that are not heated before consumption, such as delicatessen sandwiches, result in risk to consumers. For instance, the data reported during 2001 to 2008 to the CDC Food-Borne Disease Outbreak Surveillance System showed that 40% (328/813) of the HuNoV outbreaks investigated implicated sandwiches, salads, or other foods eaten raw or lightly cooked (8). A review by Todd and coworkers (9) revealed that HuNoV-associated food-borne gastroenteritis outbreaks are frequently linked to food handlers. In over 60% of the 376 reviewed outbreaks, direct food handling by an infected person or carrier of HuNoV was associated with the spreading of these outbreaks. Furthermore, in almost 30% of the HuNoV outbreaks analyzed in the study, food handlers did not wear gloves while preparing the foods, contrary to the recommendations by the Codex Alimentarius (10). Inadequate hand hygiene and gloving seem, therefore, to play major roles in HuNoV transmission linked to food handlers.

Recent studies have indicated that HuNoV is transmitted efficiently between hands, food items, and environmental surfaces during donor surface-recipient surface interaction (11). Models for simulating the transmission of HuNoV during food preparation have been developed (12, 13), but information on the transmission routes and quantities of HuNoV transferred during the actual food preparation events, such as manual preparation of RTE foods, is still limited. With more accurate knowledge of the transmission routes of HuNoV, intervention measures, such as changing gloves, can be targeted more efficiently.

In the present study, our objective was to determine whether and to what extent HuNoVs or their surrogate, murine noroviruses (MuNoVs), are transferred from virus-contaminated hands (or underneath gloves in the case of HuNoV) to clean latex gloves during gloving. The second objective was to determine the transmission of HuNoV or MuNoV, either from the food ingredient (cucumber; *Cucumis sativus* L.) or the food handlers' hands, by simulating manual preparation of a cucumber sandwich. Virus transfer was investigated by swabbing donor and recipient surfaces with polyester swabs. Environmental and food surfaces were monitored for viruses with reverse transcription-quantitative PCR (RT-QPCR). Using a predictive transfer model, the lowest

Received 8 April 2014 Accepted 18 June 2014
Published ahead of print 20 June 2014
Editor: D. W. Schaffner
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doi:10.1128/AEM.01162-14

level of HuNoV contamination on hands that would lead to transfer of at least one infective virus particle from hands to gloves and to the final product was estimated. The quantity of the sandwich servings onto which HuNoV could be transferred from either virus-contaminated hands or food ingredients via the gloves of the food handler was also calculated.

MATERIALS AND METHODS

Viruses. For artificial contamination of powder-free latex gloves [manufactured according to standard D3578-05(2010), Standard Specification for Rubber Examination Gloves] or cucumber surfaces, we used murine norovirus 1 (MNV-1), which was obtained from Herbert W. Virgin at the Washington University School of Medicine (St. Louis, MO, USA), or a human stool preparation containing HuNoV genogroup II cluster 4 (GII.4).

HuNoV. A 10% fecal suspension was prepared from the stool containing the HuNoV GII.4 in phosphate-buffered saline (PBS) (pH 7.2), cooled to 5°C for 2 h, and frozen at −70°C in aliquots. A standard curve was plotted for serial 10-fold dilutions of RNA, and one RT-QPCR-detectable virus unit (pcr-u) was defined as the highest 10-fold dilution of the sample showing a positive result with a cycle threshold (C_T) of <40 (14). The endpoint dilution from the lowest dilution of the sample to the first dilution of the sample giving a negative result in RT-PCR revealed a virus concentration of 10 $\log_{10}$ pcr-u/ml in RT-QPCR, equal to 9.7 $\log_{10}$ genome copies/ml.

MuNoV. MuNoV was cultured in RAW 264.7 cells (American Type Culture Collection [ATCC] CRL-2278) in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), 10 mM HEPES (Sigma-Aldrich, St. Louis, MO, USA), and 1% glutamine-penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA). After the viruses had been cultivated on confluent RAW 264.7 cell monolayers for 2 to 3 days, the infected cells were subjected to freezing and thawing three times to release the viruses. The titer of MuNoV released from the cells was determined to be approximately 7 $\log_{10}$ PFU/ml by viability assay (15). The PCR titer of the MuNoV stock was defined as 10 $\log_{10}$ pcr-u/ml. A standard curve was plotted for serial 10-fold dilutions of RNA, and one RT-QPCR-detectable pcr-u was defined as the highest 10-fold dilution of the sample showing a positive result with a C_T of <40.

Preparations for trials. All trials were performed in a class I biosafety cabinet on a disposable cover (Nalgene Versidry; VWR International, Radnor, PA, USA). Disposable latex gloves were used in the trials (SemperGuard latex IC; Sempermed, Clearwater, FL, USA), straight from a package. Knives, made entirely of stainless steel, were washed and then sterilized in an autoclave before use and between the trials. Plastic pipette tip box (ART; Thermo Fisher Scientific, San Diego, CA, USA) covers (7.5 by 12.5 by 3 cm), made of polypropylene, were used as surrogates for slices of bread. They were used to enable detection of viruses by a swabbing method. These plastic box covers, referred to below as "bread," were washed with soap and water before use and were discarded after every trial. The cucumbers were washed with tap water, dried on a paper towel, and slit vertically before beginning the experiments. One volunteer, referred to as the test person, who was part of the research group, was used in the transfer studies. The hands of the test person were washed with soap and water and allowed to dry before inoculating MuNoV on them or donning latex gloves for inoculation of HuNoV. All items needed in the trials were placed in a biosafety cabinet before beginning the experiments.

Transfer of MuNoV and HuNoV while donning latex gloves. The transfer of MuNoV from artificially contaminated hands to clean latex gloves was tested as follows. MuNoV (6 $\log_{10}$ pcr-u) was inoculated on the right or left clean bare hand of a single test person from the research group. The 100-μl dose of virus was spread evenly on every fingertip and on the palm of the left or right hand. The virus was allowed to dry on the hand at room temperature for 60 min, during which time the test person was not allowed to use the inoculated hand. After the incubation period, the test person performed the gloving. Gloving was performed the same way in every trial: the test person took the gloves from a container with the right hand and then donned the gloves, first on the left and then on the right hand. After gloving, swab sampling was immediately performed from the outside of the gloved left and right hands separately, using a polyester swab (175KS01; Mekalasi Oy, Nurmijärvi, Finland) moistened in glycine buffer, pH 9.5, according to the protocol described by Rönnqvist and coworkers (16).

The transfer of HuNoV (6 $\log_{10}$ pcr-u) from hands to gloves was performed in a similar manner, with two differences. First, for safety, HuNoV was inoculated on a latex glove, not a bare hand, after which the test person donned a clean pair of gloves. Second, the virus transfer during gloving was not only tested after a drying period of 60 min postinoculation, but also as wet without drying.

MuNoV and HuNoV transfer during manual preparation of the delicatessen sandwich. To test virus transfer between surfaces in the process of manually preparing a cucumber sandwich, the test person performed the preparation, after which the food and environmental surfaces were swabbed. An inoculation dose of 3.5 $\log_{10}$ pcr-u (100 μl) of MuNoV or HuNoV was seeded on the test person's latex-gloved right or left hand evenly as droplets across the entire surfaces of the hands (palms and fingertips) or on half of a cucumber (the top half of the outer surface of the cucumber lying horizontally). After an incubation period of 60 min at room temperature (21 ± 1°C), the preparation was performed as follows: (i) the right-handed test person grasped the cucumber with the left hand; (ii) took the knife into the dominant right hand; (iii) cut six slices of the cucumber, each 5 mm thick and 40 mm in diameter; and (iv) placed the slices on top of the bread with the right hand.

Swab samples were taken from the following surfaces: (i) palm and fingers of the glove of the right hand, (ii) palm and fingers of the glove of the left hand, (iii) the entire knife for cutting the cucumber, (iv) the outer surface of the cucumber, (v) the inner and outer surfaces of cucumber slices placed on the bread, and (vi) the top and sides of the bread. Surface sampling was performed with polyester swabs moistened in glycine buffer (pH 9.5) by carefully swabbing the entire area of the target surfaces. All the trials were performed three times. After sampling, the swabs were processed directly.

Virus elution, RNA extraction, and RT-QPCR. A semidirect lysis method was used to elute the viruses and to prepare the sample for RNA extraction, according to the method of Rönnqvist and coworkers (16). Briefly, the viruses were eluted from the swabs, first with 2 ml of glycine buffer, pH 9.5, and after an incubation of 10 min in an orbital shaker (IKAKS 2060 basic; Patterson Scientific Camlab Ltd., Cambridge, United Kingdom) at 250 rpm, 4 ml NucliSens miniMag (bioMérieux, Boxtel, The Netherlands) lysis buffer was added. After the second 10-min incubation, RNA extraction was performed.

RNA extraction was performed according to the instructions for the NucliSens miniMag kit (input volume, 6 ml). Amplification of MuNoV and HuNoV were performed, using a TaqMan RT-QPCR for the polymerase-gene-capsid-gene junction, according to the protocols recently described by Rönnqvist and coworkers (16). In brief, the detection was performed using a QuantiTect Probe RT-PCR kit (Qiagen, Venlo, The Netherlands) with the Rotorgene 3000 detection system (Corbett Life Science, Sydney, Australia), using the primers MNVfor and MNVrev and the probe MNV for MuNoV (17) and COG2R (−) and QNIF2d (+) and the probe QNIFS (+) for HuNoV (18).

Standard curves were prepared from 10-fold serial dilutions of MuNoV and HuNoV RNAs in water (starting concentration, 10 $\log_{10}$ pcr-u/ml), which were analyzed simultaneously with the samples and used to calculate the pcr-u counts of the samples. Duplicates of RNA samples, a negative control for RNA extraction, a negative PCR control containing distilled water, and a nontemplate control were included in every PCR run.

Virus recovery rate and transfer coefficient calculations. The remnant recovery rates were calculated as the observed pcr-u counts of the

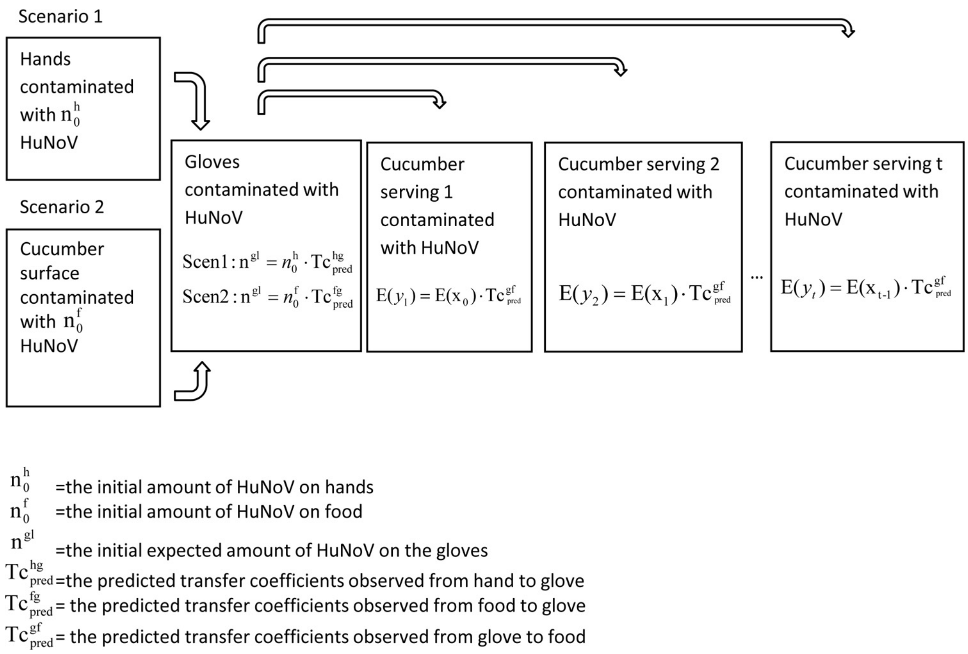

FIG 1 Model for evaluating the extent of HuNoV contamination by a food handler's gloved hand or by a food ingredient during manual preparation of a cucumber sandwich.

donor surfaces (left hand, right hand, and outer surface of the cucumber) divided by the observed original pcr-u count of the inoculation dose multiplied by 100%. The cucumber slices were regarded as donor surfaces when the cucumber was inoculated with the viruses, because the virus was inoculated onto the same area where the slices were cut. The transfer coefficients were calculated similarly by comparing the pcr-u count of the acceptor surface with the observed original pcr-u count of the inoculation dose, which was incubated in the test tube for the same amount of time as the samples on the donor surfaces. If the pcr-u count of the acceptor surface was not positive in all repetitions, the pcr-u counts of the negative samples from acceptor surfaces were included in the calculations as 0 pcr-u. The pcr-u counts in this study were normalized over the initial pcr-u count of the virus inoculation dose (3.5 $\log_{10}$ pcr-u per 100 μl for MuNoV and HuNoV). Since one 100-μl inoculation dose was included as a sample in every test series and the virus recovery rates were always calculated in relation to that sample, normalization did not impact the virus recovery rates. The estimations for true transfer coefficients were calculated from the observed transfer coefficients by the following formula: transfer × (100/observed virus recovery rate of the acceptor surface). The observed virus recovery rates of the acceptor surfaces (latex, 33% ± 10%; plastic, 27% ± 8%; stainless steel, 62% ± 13%; and cucumber, 22% ± 7%), established under the same experimental setup/conditions published previously (16), were used in these calculations.

The HuNoV and MuNoV transfer coefficients were analyzed statistically with Student's *t* test in SPSS software (SPSS Statistics; IBM). The significance was determined at a *P* value of <0.05.

Data for the statistical model. The data for the statistical model consisted of the estimates for true MuNoV and HuNoV transfer efficiencies calculated from the raw pcr-u transfer data. The trial was repeated in three categories according to the direction of virus transfer: from hands to gloves, from gloves to food ingredients, and from food ingredients to gloves. Eleven trials were performed for the hands to gloves, five trials for the food ingredients to gloves, and six for the gloves to food categories. The transfer results obtained with dried HuNoV from hands to gloves were used only to describe more accurately the conditions during the preparation of RTE food. The following assumptions were made prior to modeling: HuNoV transferred to the glove during gloving came in contact with the cucumber when the sandwich was prepared, one contaminated food ingredient (half of a cucumber) was designated one contaminated RTE food serving, the food serving was considered contaminated if at least one infective virus particle was transmitted to the food, and there was no direct contact or transfer of HuNoV between cucumbers. MuNoV and HuNoV transfer data were combined in the model based on the finding that no statistical difference was found for the transfer coefficients and recoveries of the two viruses.

Statistical model. The following Bayesian statistical model is available by request. The computations of the model were performed using OpenBUGS software (http://www.openbugs.net/w/FrontPage).

The aim of the modeling was to evaluate the extent of HuNoV contamination in the prepared cucumber sandwiches and their contact surfaces when the virus contamination originated either from the hands of the food handler or from a single food ingredient. We assumed that the transfer coefficients (Tcs) observed from hand to glove during the glove changing (Tc^{hg}), from glove to food ingredient during contact (Tc^{gf}), and from food ingredient to glove during contact (Tc^{fg}) followed a beta distribution (data model):

$$\begin{aligned} Tc_i^{hg} &\sim \mathrm{beta}(\alpha_1, \beta_1),\ i = 1, \ldots, 11 \\ Tc_j^{gf} &\sim \mathrm{beta}(\alpha_2, \beta_2),\ j = 1, \ldots, 6 \\ Tc_k^{fg} &\sim \mathrm{beta}(\alpha_3, \beta_3),\ k = 1, \ldots, 5 \end{aligned} \tag{1}$$

where i, *j*, and *k* denote the number of trials and the Tcs are observed proportions in the trials. A conventional uninformative exponential (0.01) distribution was used as a prior for both parameters of the beta distributions.

The predicted transfer coefficient from a food handler to food was $Tc_{pred}^{hg} \times Tc_{pred}^{gf}$ (the probability that a single virus moves from a food handler to food), where the values of Tc_{pred}^{hg} and Tc_{pred}^{gf} were simulated from their posterior predictive distributions based on the observed Tcs. The predicted transfer coefficient from food ingredient to food was $Tc_{pred}^{fg} \times Tc_{pred}^{gf}$, in which the values of Tc_{pred}^{fg} and Tc_{pred}^{gf} were similarly simulated from their posterior predictive distributions.

Next, we modeled the predicted number of HuNoV-contaminated food servings after repeatedly preparing cucumber sandwich servings in two scenarios (Fig. 1). In the first scenario, the hands of the food handler were as-

TABLE 1 Virus transfer coefficients from MuNoV-contaminated hands or gloved hands inoculated with HuNoV to a clean pair of latex gloves when donning the gloves[a]

Virus	Inoculation site	Drying time (min)	Hand	Virus concn ($\log_{10}$ pcr-u/ml)	Transfer coefficient (%) (no. positive/total)	Calculated transfer coefficient (%)[b]
MuNoV	Left hand	60	Left	5.6 ± 5.2	1.5 ± 0.5 (3/3)	4.4 ± 1.5
		60	Right	6.0 ± 5.2	2.6 ± 3.2 (3/3)	7.8 ± 9.6
	Right hand	60	Left	0.0 ± 3.0	0.0 ± 0.0 (1/3)	0.0 ± 0.0
		60	Right	4.5 ± 4.4	0.1 ± 0.1 (3/3)	0.3 ± 0.2
HuNoV	Left gloved hand	0	Left	6.0 ± 5.9	11.4 ± 8.5 (3/3)	34.6 ± 25.7
		0	Right	5.3 ± 5.3	2.1 ± 2.4 (2/3)	6.5 ± 7.2
		60	Left	4.0 ± 2.9	0.1 ± 0.0 (3/3)	0.3 ± 0.0
		60	Right	4.2 ± 4.0	0.1 ± 0.1 (2/3)	0.4 ± 0.3
	Right gloved hand	0	Left	6.5 ± 5.9	32.4 ± 8.6 (3/3)	98.0 ± 26.0
		0	Right	6.0 ± 5.5	8.7 ± 5.6 (3/3)	26.2 ± 17.0
		60	Left	5.7 ± 5.5	3.6 ± 3.6 (3/3)	11.0 ± 10.9
		60	Right	4.4 ± 4.5	0.2 ± 0.3 (2/3)	0.7 ± 1.0

[a] The inoculation dose of MuNoV and HuNoV was 6 $\log_{10}$ pcr-u.
[b] Estimate of the true transfer coefficient. In estimation calculations, a following recovery rate of 33% from the surface of latex gloves was used.

sumed to initially contain 1 to 4 $\log_{10}$ virus particles before gloving and preparing a series of sandwich servings. In the second, the first single food ingredient (cucumber) that the food handler touched before preparing a series of sandwiches was assumed to contain, likewise, from 1 to 4 $\log_{10}$ virus particles. The amount of HuNoV on the gloves was assumed to decrease during every contact, so that the expected number of virus particles remaining on the gloves after the preparations with the same gloves, $E(x_t)$, was

$$E\left(x_t|n^{\text{gl}}, Tc_{\text{pred}}^{\text{gf}}\right) = n^{\text{gl}} \times e^{\left[t \cdot \log\left(1-\text{Tc}_{\text{pred}}^{\text{gf}}\right)\right]} \quad (2)$$

where n^{gl} is the initial expected amount of HuNoV on the gloves, transferred either from the hands or from an initial single food ingredient, $\text{n}_0^{\text{h}} \times \text{Tc}_{\text{pred}}^{\text{hg}}$ or $\text{n}_0^{\text{f}} \times \text{Tc}_{\text{pred}}^{\text{fg}}$, depending on the scenario chosen (1 or 2, respectively). The expected number of viruses in the next food serving, y_{t+1}, is then

$$E\left(y_{t+1}\right) = E\left(x_t\right) \times \text{Tc}_{\text{pred}}^{\text{gf}} \quad (3)$$

RESULTS

Transfer of MuNoV and HuNoV during gloving. The transfer of MuNoV from either the left or right hand to latex gloves during gloving by a right-handed person was investigated by testing swabs taken from gloves for the presence of MuNoV RNA by RT-QPCR. These swabs repeatedly tested positive, overall, 10/12 times (Table 1), indicating the transfer of MuNoV. When the nondominant left hand was contaminated by the virus, MuNoV RNA was detected on the glove swabs in 6/6 experiments, whereas when the dominant right hand was artificially contaminated, MuNoV RNA was detected on the gloves in 4/6 experiments. The transfer coefficients of MuNoV RNA to gloves varied from 0.1% to 7.0% when the left hand was contaminated with virus and from 0.0% to 0.2% when the right hand was contaminated. In calculating the estimates of true transfer coefficients of MuNoV, we considered the individual recovery rates from latex, plastic, stainless steel, and cucumber surfaces, obtained in a previous study with the same sampling protocol (16). Calculations of the estimations for true transfer coefficients of viruses revealed the difference in transfer coefficients between the contaminated hands, although it could not be verified statistically. In the case of the contaminated left hand, the average true transfer coefficient, calculated from a virus recovery rate of 33% obtained in the previous study (16) for both recipient gloved hands, was 6.1% ± 5.6%, whereas when the right hand was contaminated, the average coefficient was only 0.2% ± 0.1%.

When HuNoV was used instead of MuNoV in the experiments, the virus was inoculated onto the gloved hands before donning a clean pair of latex gloves (Table 1). This time, the viruses were either dried on the gloves for 60 min before gloving or the right-handed person donned clean gloves immediately after the inoculation. HuNoV RNA was transferred from the gloved hands to clean gloves as effectively as MuNoV was transferred from hands to gloves: 10/12 times when dried for 60 min and 11/12 times when wet. The virus was transferred to the gloves 10/12 times when the left gloved hand had been artificially contaminated and 11/12 times when the right gloved hand was contaminated. The transfer coefficients of HuNoV varied from 0.0% to 44.4% regardless of the drying time or the inoculation site (left or right hand), but the average concentration of viruses on the swabbed gloves (6.1 $\log_{10}$ pcr-u per hand) was higher ($P < 0.05$) when the virus inoculation remained wet than when it was left to dry for 60 min (5.0 $\log_{10}$ pcr-u per hand). No statistical difference in the transfer coefficients was observed between the HuNoV and MuNoV results in these trials.

Transfer of MuNoV and HuNoV between contact surfaces in manual preparation of a delicatessen sandwich. MuNoV transfer between a donor surface (left-hand glove, right-hand glove, or outer surface of a cucumber) and acceptor surfaces was investigated in the process of simulating manual preparation of a cucumber sandwich (Fig. 2). In calculating the remnant recovery rates of raw data from donor surfaces after the simulation experiment, we observed that the average remnant recovery rate from artificially virus-contaminated cucumber surfaces was higher (18.4%) than that from contaminated glove surfaces (5.8% and 6.6% for the left and right hand, respectively). The remnant MuNoV recovery rates from cucumber surfaces (3.0% to 48.9%) varied more than those from left- and right-hand gloves (0.4% to 13.7%) (Table 2). During the simulation study, we observed that MuNoV was transferred to more acceptor surfaces if the virus contamination had occurred on the surface of the cucumber than if it had occurred on either of the hands of the food worker (Table 2). However, we observed MuNoV transfer from the donor surface only to the first

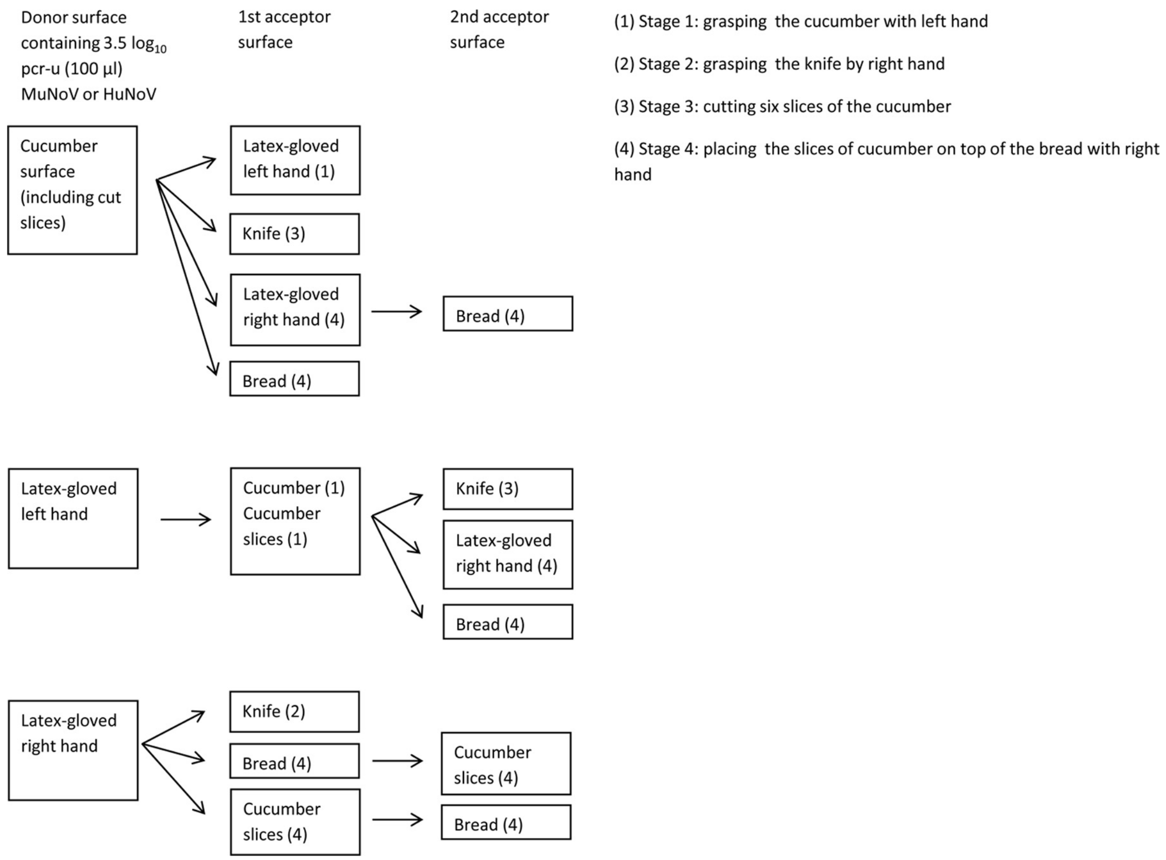

FIG 2 Possible contamination routes of MuNoV and HuNoV and stages 1 to 4, during which the contamination may occur when preparing a delicatessen sandwich.

contact surface, but not to the second, on each repetition of the simulation. In independent experiments, we observed MuNoV transfer from the artificially contaminated cucumber surface to the gloved hand holding the cucumber in 3 out of 3 experiments and to the knife blade used for cutting the cucumber in only 1 out of 3 experiments. From the contaminated left hand, we observed virus transfer to the cucumber in all three experiments. MuNoV transfer from the right gloved hand to the knife handle was observed in all three experiments, while transfer from hand to bread was observed in 2 out of 3 experiments. MuNoV was transferred more efficiently from the glove to the surface of the cucumber than from the glove to the knife handle ($P < 0.05$). Virus transfer was also more efficient from the glove to the cucumber than vice versa, although this was not supported statistically. Indeed, the highest transfer coefficient, 1.2% ± 0.6%, was observed when the virus was transferred from the glove to the cucumber surface. When estimates of the true transfer coefficients from the glove to the cucumber surface were calculated, the coefficient was even more pronounced (5.4% ± 3.1%), although its difference from other transfer events was not statistically significant. The virus transfer coefficients from the glove to the knife were low, even when the surface-specific recovery rate of the knife was taken into account in the calculations: less than 1% in all the repetitions.

The average remnant recovery rates of HuNoV from the cucumber surface (6.6%) and gloves (10.6% and 8.5% for the left and right hand, respectively) were more alike than the corresponding rates for MuNoV (Table 2). There was also less variation in the recovery rates between the trials—2.9% to 13.0% for the cucumber surface and 6.4% to 15.6% for the gloves—than in the MuNoV trials. In the transfer experiments, HuNoV transferred from the donor surfaces to the acceptor surfaces in quantities similar to those of MuNoV. We observed that HuNoV was transferred to more acceptor surfaces from the cucumber surface than MuNoV. In addition to the left gloved hand (3 out of 3 experiments) and knife blade (1 out of 3 experiments), we observed transfer to the right gloved hand in 1 out of 3 experiments and to the bread in 1 out of 3 experiments. From the left gloved hand, the virus was transferred to the cucumber in 3 out of 3 experiments and from the right-gloved hand to the knife handle in 2 out of 3 experiments. As in the MuNoV tests, more viruses were transferred from glove to cucumber than vice versa, although this could not be confirmed statistically.

Statistical model. In the Bayesian analysis, data from both the gloving experiment and the food-handling study were used in modeling. As a result of this analysis, we estimated that HuNoV contamination on the hands should be more than 3.4 $\log_{10}$ infective virus particles to result in contamination of a single prepared cucumber sandwich serving (probability, 50.0%). With 4.2 $\log_{10}$ virus particles on the hands, the probability of the sandwich becoming contaminated would already have risen to 70.0%.

In this analysis, we also calculated that HuNoV on food handlers' contaminated hands/gloves would be transferred to far more cucumber sandwich servings than by sporadic HuNoV contamination of a single food ingredient. We calculated that if 3 $\log_{10}$

TABLE 2 Virus remnant recovery rates, transfer coefficients, and estimated true transfer coefficients of MuNoV and HuNoV between surfaces in manual preparation of a cucumber sandwich after inoculation of 3.5 $\log_{10}$ pcr-u (5.5 $\log_{10}$ pcr-u/ml) of MuNoV or HuNoV on cucumber, right hand, or left hand

Virus	Inoculation site	Surface	Virus concn ($\log_{10}$ pcr-u/ml)	Remnant recovery rate (%) (no. positive/total)	Transfer coefficient (%) (no. positive/total)	Calculated transfer coefficient (%)[a]
MuNoV	Cucumber	Cucumber	4.7 ± 4.8	18.4 ± 26.4 (3/3)		
		Right hand	<1[b]			
		Left hand	3.3 ± 3.1		0.7 ± 0.5 (3/3)	2.1 ± 1.6
		Knife	1.7 ± 1.9		0.0 ± 0.0 (1/3)	0.3 ± 0.5
		Cucumber slices	2.9 ± 2.4	0.3 ± 0.1 (3/3)		
		Bread	<1			
	Left hand	Cucumber	3.6 ± 3.1		1.2 ± 0.6 (3/3)[d]	5.4 ± 3.1
		Right hand	NC[c]			
		Left hand	4.2 ± 4.1	5.8 ± 5.7 (3/3)		
		Knife	<1			
		Cucumber slices	<1			
		Bread	<1			
	Right hand	Cucumber	NC			
		Right hand	4.3 ± 4.2	6.6 ± 6.1 (3/3)		
		Left hand	NC			
		Knife	2.9 ± 2.7		0.2 ± 0.2 (3/3)[d]	0.4 ± 0.3
		Cucumber slices	<1			
		Bread	0.0 ± 1.6		0.0 ± 0.0 (2/3)	0.1 ± 0.1
HuNoV	Cucumber	Cucumber	4.2 ± 4.2	6.6 ± 4.7 (3/3)		
		Right hand	1.9 ± 2.1		0.0 ± 0.1 (1/3)	0.1 ± 0.2
		Left hand	2.9 ± 2.7		0.5 ± 0.4 (3/3)	1.4 ± 0.7
		Knife	3.3 ± 0.3		0.4 ± 0.1 (1/3)	0.1 ± 0.1
		Cucumber slices	2.3 ± 2.5	0.6 ± 0.3 (3/3)		
		Bread	1.9 ± 2.1		0.0 ± 0.1 (1/3)	0.2 ± 0.2
	Left hand	Cucumber	3.7 ± 3.8		1.5 ± 1.9 (3/3)	6.9 ± 8.8
		Right hand	NC			
		Left hand	4.3 ± 3.7	10.6 ± 3.6 (3/3)		
		Knife	<1			
		Cucumber slices	<1			
		Bread	<1			
	Right hand	Cucumber	NC			
		Right hand	4.3 ± 3.7	8.5 ± 2.3 (3/3)		
		Left hand	NC			
		Knife	2.7 ± 2.8		0.3 ± 0.2 (2/3)	0.4 ± 0.5
		Cucumber slices	<1			
		Bread	<1			

[a] Estimate of the true transfer coefficient. In estimation calculations, the following recovery rates were used: outer surface of cucumber, 22%; surface of plastic, 27%; surface of stainless steel, 62%; and surface of latex gloves, 33%.
[b] Under the detection limit of 0.1 $\log_{10}$ pcr-u.
[c] NC, no contact with virus.
[d] More efficient transfer from the glove to the surface of the cucumber than from the glove to the knife handle ($P < 0.05$).

HuNoV particles were present on the hands of the food handler before gloving and food preparation, the probability of the expected value for HuNoVs being 1 or more in the 8th cucumber sandwich serving would still be over 50% (Fig. 3). If, however, the same number of particles were present on the surface of a cucumber, the probability of transfer to even the first serving would be less than 5%. According to the calculations, the HuNoV level on a single contaminated cucumber would have to be 3.7 $\log_{10}$ virus particles to have a probability of virus transfer similar to that for hands containing 3 $\log_{10}$ HuNoV particles.

DISCUSSION

The importance of HuNoVs as the causative agents of food-borne gastroenteritis outbreaks has been understood for several decades (1). The contamination routes of these viruses, however, are still being investigated with the aid of novel techniques. In the present study, the transfer of HuNoVs and their surrogate MuNoVs was measured for the first time during gloving and simulated preparation of a sandwich. Subsequently, the data were used in a model to calculate the number of servings contaminated as a result of transfer between hands and surroundings, demonstrating that contaminated gloves transferred HuNoV to the food servings more efficiently than sporadic food ingredient contamination during handling.

In the present study, the transfer coefficients of both MuNoV and HuNoV RNA or virus from glove to cucumber were suggested to be higher than the transfer coefficients from cucumber to glove, although the difference could not be verified statistically. Previously, a similar finding was observed for feline calicivirus (FCV), which was transferred more efficiently (46%) from fingertips to ham than vice versa (6%) (19). More recently, HuNoVs were

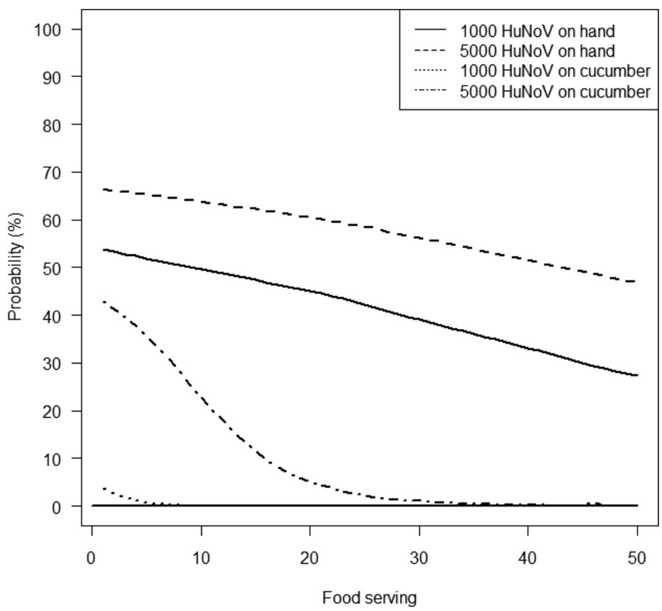

FIG 3 Probabilities of the expected value of HuNoV particle transfer from contaminated hands of a food handler or from a contaminated single cucumber food ingredient to food servings being ≥1 in a food serving when the initial numbers of HuNoVs on the hands and on the cucumber are 3 $\log_{10}$ and 3.7 $\log_{10}$ virus particles.

transferred more efficiently from gloves to food ingredients (lettuce [*Lactuca* L.], 2.7%; ham, 16.2%) than from gloves to stainless steel (0.1%) (20). This is in agreement with the present study, in which HuNoV and MuNoV were transferred more efficiently from gloves to cucumber than from gloves to a stainless steel knife. The transfer coefficients were similar, despite differences in the virus concentrations used: 5.5 $\log_{10}$ PFU per surface for an inoculation dose of FCV (19), 6.8 $\log_{10}$ genomic copies of HuNoV or 4.3 $\log_{10}$ PFU of MuNoV per surface (20), or 6 $\log_{10}$ pcr-u of HuNoV and MuNoV in the gloving experiment and 3.5 $\log_{10}$ pcr-u of HuNoV and MuNoV in the present food-handling study. Stals and coworkers (20) showed that HuNoV and MuNoV were transferred from gloves to sandwich bun. Similarly, we observed in our study that MuNoVs were sometimes transferred from gloves to bread, even though the transfer coefficient obtained cannot be considered accurate because of the structural differences between actual bread and the plastic surface we used as a model. Tuladhar and coworkers (21) observed that the transfer coefficients of MuNoVs from bare fingertips to cucumbers were approximately 50%, much higher than our estimate of the true transfer of MuNoV from gloves to cucumber, 5.4%. Although we had different settings, since in our study the virus was distributed to the entire palm of the hand instead of a fingertip, it may be that bare fingers transfer noroviruses more efficiently than latex gloves. No statistical difference was found for the transfer coefficients and remnant recovery rates of MuNoV and HuNoV in the present study or in the study by Sharps and coworkers (22), suggesting that MuNoV serves as a suitable surrogate for HuNoV in virus transfer studies.

Surface materials affect the recovery rates of HuNoV obtained by swabbing (20, 23). Therefore, in the present study, estimates of the true transfer coefficients of MuNoV and HuNoV were calculated. The highest single remnant recovery rate from a cucumber surface for MuNoV, 49%, was similar to the HuNoV recovery rate of 32% (inoculation dose, 4.3 $\log_{10}$ pcr-u) from cucumber reported by Scherer and coworkers (23) and the 50% MuNoV recovery rate from the lettuce surface obtained by Stals and coworkers (20). This indicates that only a small portion of MuNoVs were transferred onward from the cucumber in that single trial. The recovery rates Stals and coworkers (20) obtained from a nitrile glove surface (38%) and the recovery rate we obtained from latex gloves without any preparation in a previous study (33%) (16) were much higher than the remnant recovery rates we obtained in this study from latex gloves for HuNoV after preparing the cucumber sandwich: 6.5% to 15.6%. This suggests that a high proportion of HuNoVs were transferred from the latex glove surfaces to cucumbers and other contact surfaces. Wang and coworkers (24) reported much lower MuNoV recovery rates, averaging 11.4%, from knives than the 62% we used in the true transfer coefficient calculations. These unequal recovery rates may have resulted from the difference in virus recovery methods: Wang and coworkers (24) reported that the viruses were eluted from the knives by stomaching. The recovery rates for viruses published in other studies seem to be comparable only when identical recovery methods are used.

Barker and coworkers (25) showed that when the initial dose of the virus on the fingertips was approximately 4.3 $\log_{10}$ pcr-u, HuNoV was transferred from contaminated fingertips sequentially to as many as seven clean melamine surfaces. This contamination level was actually obtained in a volunteer study by Liu and coworkers (26): the HuNoV levels on the rinse samples of the hands of six HuNoV-infected volunteers ranged from 2.81 to 4.45 $\log_{10}$ genomic equivalent copies. In the present study, 4 $\log_{10}$ pcr-u or larger amounts of HuNoV on contaminated hands were estimated to lead, despite covering of the hands with gloves, to contamination of essentially all the sandwich servings prepared after gloving on the same working shift. In the transfer model used in the present study, a sandwich serving was defined as contaminated when at least one HuNoV genome was transferred to the sandwich. The definition is based on the calculations of Teunis and coworkers (27), who estimated the probability of even one HuNoV infecting a human as being 50%. Although RT-QPCR cannot discriminate between infectious and noninfectious particles transferred between hands, gloves, and food products during sandwich preparation, the estimate gives direct information on the risk of sandwich contamination with infective viruses during food preparation. The quantitative exposure model of Mokhtari and Jaykus (13) and the recent HuNoV transfer model of Verhaelen and coworkers (28) lend support to the concept that hands are a significant vehicle in HuNoV transmission during the processing of RTE foods, in line with the present study.

Protective gloves are considered to aid in preventing the transfer of food-borne viruses during food preparation (10). However, in the present study, contamination of hands with MuNoV and HuNoV prior to gloving led to virus contamination of the protective gloves in the majority of experiments. If infective, enough HuNoVs could transfer to the cucumber sandwiches prepared and cause infection when consumed. The present study supports the findings by Mokhtari and coworkers (13) that proper hand washing prior to gloving would result a significant drop in virus levels on the hands, thus preventing transfer of HuNoV from hands to RTE foods more efficiently than use of only one of these prevention measures. Recently, the Codex Alimentarius guidelines on the application of general principles of food hygiene to the control of viruses in food was adopted by the Codex Alimentarius

Commission (29). The document clearly states that wearing gloves or the use of hand sanitizers does not exempt food handlers from having thoroughly washed their hands before donning gloves. Such practices require good compliance and are dependent on, among other factors, education and facilities.

The present study has demonstrated that HuNoV is transferred broadly both from food ingredients to the environment and from food handlers' hands to food ingredients and to prepared RTE foods. Contamination of fresh food ingredients by HuNoV during crop production can be reduced by using clean water for irrigation of the crops and washing. Our study showed that wearing gloves reduces the risk of virus transfer from contaminated hands and can partially, but not completely, protect foods from contamination by the food handler. Therefore, effective hand hygiene, including hand washing with soap and water, is crucial in preventing contamination of otherwise HuNoV-free food ingredients by these viruses.

ACKNOWLEDGMENTS

This work was part of the project Detection and Elimination of Viruses from Processing Environments (462002) funded by Tekes (National Technology Agency of Finland) and several Finnish companies.

We thank Satu Oristo for her technical assistance in the laboratory and Ingeborg Boxman for her indispensable help during the writing process.

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